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6. AUTHOR(S)
L. W. Parks

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
North Carolina State University
Box 7003
Raleigh, NC 27695-7003

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
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13. ABSTRACT *(Maximum 200 words)*

Virtually every antifungal agent in use intercedes some aspect of sterol synthesis or function. Ergosterol is the principal sterol in fungi, while cholesterol is the most abundant sterol in vertebrates. Research in our laboratory has shown that the structural differences in ergosterol, in comparison to cholesterol, have distinctive biochemical and physiological effects in fungi.

Under the completed contract we have shown that as the sterol composition of sterol auxotrophic strains of *Saccharomyces cerevisiae* is altered there is disturbance of the mating efficiency of the strains. The normal sterol, ergosterol, mediates a 30-fold higher productive mating efficiency in the auxotrophs than when the cells are supplied with stigmasterol. Using electron and visible microscopy, we have shown that the mated pairs in stigmasterol remained adherent but prezygotic even after 12 hours incubation. Ergosterol rescued the cells and permitted zygote formation.

Based on those experiments it was clear that membrane fusion was perturbed by sterol alterations. Continuing work with the renewal grant is focusing on membrane fusion in various normal cell biological processes.

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Yeast, antifungal agents, membranes, lipids, ergosterol

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FINAL REPORT

Title: Biochemically vulnerable sites for antifungal intercession in the control of fungal growth.

Contract: DAAL03-89-D-0003-07

Period of contract: 01-05-91 to 30-04-94

Report: Fungal biodeterioration accounts for millions of dollars in damage to stored material and food-stuffs annually. In addition, the production by fungi of secondary metabolites such as aflatoxins, tricothecenes, ergot and clavine alkaloids, destroy or make unacceptable large quantities of stored materials such as various grains. Yet, there are no effective controlling agents for fungi under those conditions. In addition to the deterioration of supplies, fungal infections in field personnel remain the most recalcitrant to therapy, seriously eroding readiness and availability. Thus, effective control of fungi under a wide variety of conditions is a high priority concern. However, all antifungal agents that are currently in use are either highly toxic, poorly soluble, or both. More suitable agents are sorely needed.

Virtually every antifungal agent in use intercedes some aspect of sterol synthesis or function. Ergosterol is the principal sterol of fungi, while cholesterol is the most abundant animal sterol. Research in our group has demonstrated that the structural differences in ergosterol, in comparison to cholesterol, have distinctive biochemical and physiological effect in the fungi. Since an ideal fungal controlling agent would perturb some essential metabolic function in the fungus without affecting animal physiology, the cited structural differences are attractive starting points for probing the unique metabolic and physiological roles for sterols in fungi. With that information in hand, it is logical that more effective and specifically targeted antifungal agents could be developed through biorational design.

Sterol auxotrophic strains of *Saccharomyces cerevisiae* were grown and allowed to conjugate on media supplemented with various sterols. The mating efficiency of the auxotrophs is perturbed by the replacement of the normal yeast sterol, ergosterol, with other sterols. After 4 h of mating, cells grown on ergosterol exhibited a 30-fold higher productive mating efficiency than those cells grown in stigmasterol. Aberrant budding by the conjugants was enhanced following incubation on stigmasterol and other non-ergosterol sterols.

Using light and electron microscopy, we demonstrated that there is a reduced ability for stigmasterol-grown cells to undergo cytoplasmic fusion during conjugation. Many of the mated pairs remained adherent but prezygotic even after 12 h of incubation. The addition of ergosterol to cells previously grown on stigmasterol rescued the organisms, allowing for zygote formation and normal budding.

Insertion of the unsaturation at C22 of the side-chain of ergosterol is one of the final reactions in the synthesis of the sterol. The reaction is important because it introduces rigidity into the side-chain, making the sterol much more refractory to intercalation with the long acyl groups of the phospholipids in membranes. Such physical effects on the membranes are

important in maintaining structural integrity and regulating the activity of various membrane functions. The desaturase reaction is therefore viewed as critical in normal sterol function and should be an additional site for biochemical intercession.

Our research was extremely frustrated because of an inability to obtain mutants totally lacking C22 desaturase activity. The structural gene for that enzyme, *ERG5*, could not be cloned by complementation until we had a reliable mutant, *erg5*, for selection of the wild-type allele. After pursuing many separate strategies we were finally successful in defining conditions that reliably gave us *erg5* mutants. With those in hand it was necessary to devise an appropriate selection criterion for isolating the prototrophs following transformation with a clone bank containing the *ERG5* allele. Very recently, we have succeeded in developing a highly reliable selective medium, and we are currently screening the suspected clones.

Substantial progress was made in pursuing the objectives of the original grant. Under the continuation of our work, we shall make additional effort in resolving the physiological roles for sterols in fungi and in defining biochemically vulnerable sites for antifungal intercession.

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Participating personnel in this project:

L. W. Parks
M. E. Tomeo
S. R. Tove

Degrees earned: None

Degree candidates with degree: M. E. Tomeo, for Ph.D.

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